THE DISINFECTANT ACTIVITY OF CAUSTIC SODA

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(With 2 Figures in the Text)

In connexion with an investigation made for the Ministry of Health into the cleaning and sterilization of milk bottles, the general results of which it is hoped to publish in a future issue of this *Journal*, it proved necessary to obtain exact information on the bactericidal activity of caustic soda. In particular, the effect of varying the concentration and the temperature of the solution on the destruction of sporing and non-sporing organisms had to be determined before it was possible to lay down the strengths at which caustic soda should be used under varying conditions of plant operation in order to bring about a given degree of sterility. Careful quantitative observations were therefore made in the laboratory.

1. Observations on *Bact. coli*

Technique

After some preliminary work, the following technique was adopted.

A non-clumping, relatively heat-resistant strain of *Bact. coli* was selected and used throughout. It was grown for 21 hr. at 37° C. on heart extract agar slopes. The growth was washed off with sterile glass-distilled water and standardized by opacity to 3000×10^6 per ml., using a standard opacity tube made by counting a suspension of *Bact. coli* from a 24 hr. agar culture in a Helber chamber adapted for dark-ground illumination (Wilson, 1922, 1926).

Four ml. of the 3000×10^6 suspension were added to 36 ml. of a sterile 1/5000 dilution in glass-distilled water of a full-cream dried milk powder, approximately equivalent to a 1/500 dilution of ordinary liquid milk. The total count of *Bact. coli* in this suspension was 300×10^6 per ml.

Five ml. of this bacterial suspension in milk were added to 5 ml. of an accurately titrated solution of caustic soda contained in 30 ml. alkali-free glass bottles fitted with metal caps. The temperature both of the bacterial suspension and of the caustic soda solution was brought to the required level before mixing by immersion of their respective containers in a water-bath.

The temperature of the water in the bath was maintained approximately constant throughout the experiment by the addition of minute pieces of ice if the temperature required was 20° C., or by the use of a bimetallic regulator if the required temperature was 30° C. or over. It was the whole-time duty of one assistant to supervise these baths during the course of an experiment, and to keep the suspensions well mixed by frequent shaking.

Viable counts were made by the roll-tube method (Wilson, 1922; Wilson, Twigg, Wright, Hendry, Cowell & Maier, 1935) on the original suspension in milk immediately the experiment was begun. As the dropping pipette technique would have been difficult with fluids at so many different temperatures, specially made straight-sided pipettes

capable of delivering 0·1 and 0·2 ml. quantities were used instead for inoculating roll tubes and for making certain dilutions. The viable count on the milk suspensions was usually about 100×10^6 per ml., making the viable count on the disinfectant mixture about 50×10^6 per ml.

Counts were made at intervals, regulated by a stop-watch, of the disinfectant mixtures. The quantity withdrawn, 0·1 or 1·0 ml., was inoculated into $N/4$ Ringer solution containing an amount of HCl just sufficient to neutralize the caustic soda. Further dilutions were made in ordinary $N/4$ Ringer solution. *Counts were made only on dilutions*, since experience showed that if the disinfectant mixture was added directly to the agar, even if this contained the requisite amount of HCl to neutralize the caustic soda, the results were very irregular. Usually two 0·1 ml. and two 0·2 ml. quantities were put up for each dilution, so that the results were based on the mean values of two to four tubes, depending on the number that were countable. All roll tubes were incubated at 37° C. and counted by one observer after 3 days.

In working out the constants of the disinfectant at different concentrations and temperatures the formulae proposed by Phelps (1911) were used. For the reaction velocity k, the formula was

$$k = \frac{1}{t} \log \frac{B}{b},$$

where t = time in minutes, B = number of viable organisms at the start, and b = number of viable organisms at the end of time t.

For the concentration coefficient n, the formula was

$$n = \log \frac{k_2}{k_1} \div \log \frac{C_2}{C_1},$$

where k_1 = reaction velocity at concentration C_1, and k_2 = reaction velocity at concentration C_2.

For the temperature coefficient θ, the formula was

$$\theta^{10° \text{C.}} = \frac{k_1}{k_2},$$

where k_1 = reaction velocity at higher temperature and k_2 = reaction velocity at lower temperature.

Results. Mean values of k, n, and θ

Reaction velocity k

The reaction-velocity constants for different strengths of caustic soda at different temperatures were worked out for each interval of time, and the mean value estimated by averaging the values for equal intervals. This yielded results which were, of course, practically identical with those obtained by taking the single value for the whole period. The figures are set out in Tables 1–6. There was some variation in the values of k between different experiments at any one concentration and temperature, probably due to imperfect standardization of all the various factors concerned. To obtain reliable averages three to seven experiments had to be carried out under each set of conditions.

The rate of disinfection of *Bact. coli* by 0·05% sodium hydroxide at 20° C. was comparatively slow, while at 40° C. it was about seven times as rapid, and approximately equal to that of 0·1% NaOH at 20° C. Fig. 1 shows the great differences in the values of k produced by relatively small changes in concentration and temperature.

Table 1. *Mean values of reaction velocity constant k for* 0·05% NaOH *at* 20° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean k
12. x. 38	0–40	7·7198	5·4575	0·057
14. x. 38	0–40	7·8016	5·1750	0·066
25. xi. 38	0–40	7·4771	5·5323	0·049
13. xii. 38	0–60	7·7853	4·3617	0·057
11. i. 39	0–60	7·7364	5·1790	0·043
17. i. 39	0–60	9·0021 6·9751	6·5260 3·0682	0·053
Total	300	38·5202	25·7055	0·325
Arithmetic mean	50	7·7040	5·1411	**0·054**

Table 2.. *Mean values of reaction velocity constant k for* 0·1% NaOH *at* 20° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean k
25. xi. 38	0–5	7·4771	5·2014	0·455
13. xii. 38	0–10	7·7853	4·0162	0·377
11. i. 39	0–12	7·7364	4·0759	0·305
20. i. 39	0–9	7·8435	4·8663	0·331
29. iii. 39	0–6	7·7023	5·2430	0·410
30. iii. 39	0–8	7·7924	4·9345	0·357
31. iii. 39	0–8	7·7216	5·0635	0·333
Total	58	54·0586	33·4008	2·568
Arithmetic mean	8·3	7·7227	4·7715	**0·367**

Table 3. *Mean values of reaction velocity constant k for* 0·05% NaOH *at* 30° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean k
20. i. 39	0–40	7·8435	3·4983	0·109
24. i. 39	0–40	7·6628	3·9644	0·092
27. i. 39	0–40	7·1501*	2·2430	0·123
31. i. 39	0–30	7·7764	4·3324	0·115
7. ii. 39	0–30	7·7559	3·4713	0·143
Total	180	38·1887	17·5094	0·582
Arithmetic mean	36	7·6377	3·5019	**0·116**

* Suspension diluted three times more than usual by mistake.

Table 4. *Mean values of reaction velocity constant k for* 0·1% NaOH *at* 30° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean k
20. i. 39	0–6	7·8435	2·9230	0·820
14. ii. 39	0–6	7·7924	4·0112	0·630
17. ii. 39	0–6	7·7076	3·7941	0·652
Total	18	23·3435	10·7283	2·102
Arithmetic mean	6	7·7812	3·5761	**0·701**

Table 5. *Mean values of reaction velocity constant k for* 0·025% NaOH *at* 40° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
3. ii. 39	0–10	7·5139	6·1903	0·132
7. ii. 39	0–20	7·7559	2·7959	0·248
17. ii. 39	0–10	7·7076	5·8382	0·187
21. ii. 39	0–20	7·7889	2·4955	0·265
29. iii. 39	0–12	7·7023	3·2337	0·372
31. iii. 39	0–8	7·7216	6·0483	0·209
Total	80	46·1902	26·6019	1·413
Arithmetic mean	13·3	7·6984	4·4337	**0·236**

Table 6. *Mean values of reaction velocity constant k for* 0·05% NaOH *at* 40° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
31. i. 39	0–10	7·7764	3·6604	0·412
3. ii. 39	0–10	7·5139	3·4830	0·403
7. ii. 39	0–10	7·7559	4·2148	0·354
14. ii. 39	0–10	7·7924	3·9112	0·388
Total	40	30·8386	15·2694	1·557
Arithmetic mean	10	7·7097	3·8174	**0·389**

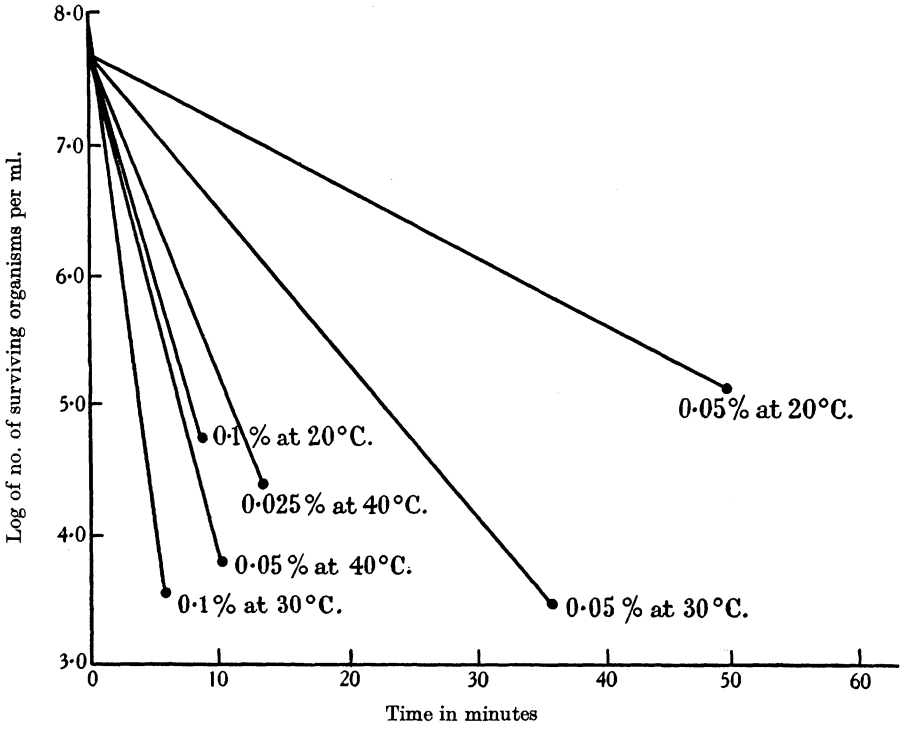

Fig. 1. Rate of death of *Bact. coli* when submitted to different concentrations of sodium hydroxide at different temperatures.

Comparison of the reaction velocity rates at successive intervals of time under a given set of conditions showed that, with the exception of 0·05% NaOH at 40° C. in which the results tended to be irregular, the value of k was always slower at the beginning of disinfection than later (Table 7). In other words, the rate of disinfection increased throughout the experiment. It is possible that if observations could have been continued till the sterility point was nearly reached, the value of k might have diminished again, giving a

Table 7. *Mean values of reaction velocity constants at different stages of the disinfection curve* NaOH *with* Bact. coli

Concentration, temperature and no. of experiments (in brackets)	Above: Interval in min. Below: Mean k			
0·05% NaOH at 20° C. (4)	0–10 0·031	10–20 0·045	20–30 0·070	30–40 0·054
0·1% NaOH at 20° C. (3)	0–4 0·286	4–8 0·389		
0·05% NaOH at 30° C. (3)	0–10 0·076	10–20 0·124	20–30 0·174	
0·1% NaOH at 30° C. (3)	0–3 0·493	3–6 0·932		
0·025% NaOH at 40° C. (2)	0–10 0·247	10–20 0·266		
0·05% NaOH at 40° C. (3)	0–5 0·491	5–10 0·311		

sigmoid type of curve instead of a straight line when the logarithms of the surviving organisms were plotted against time. There was a suggestion of this in the experiments with 0·05% NaOH at 20° C., when the average value of k rose from 0·031 in the first 10 min. to reach a maximum of 0·070 between 20 and 30 min., and then fell again to 0·054 in the 30–40 min. period. Unfortunately, owing to the technical difficulties referred to above of making satisfactory observations on the undiluted disinfectant mixture, it proved impossible with the higher concentrations and temperatures to follow the reactions far enough to determine whether the diminution in the value of k in the later stages of disinfection was a general phenomenon or not.

One experiment was carried out to see if the rate of disinfection was appreciably affected by the number of organisms in the suspension. Using 0·05% NaOH at 20° C. counts were made at 20 min. intervals on two suspensions of *Bact. coli*, one containing 10 million and the other 1000 million per ml. viable bacilli at the start. The results, which are given in Table 8, show that destruction occurred rather more rapidly with the weaker than with the stronger suspension.

Table 8. *Mean values of reaction velocity constants for* 0·05% NaOH at 20° C. *with different numbers of* Bact. coli

	k			
Size of inoculum	0–20 min.	20–40 min.	40–60 min.	Mean k
10×10^6 per ml.	0·049	0·075	0·072	**0·065**
1000×10^6 per ml.	0·039	0·036	0·049	**0·041**

A few experiments were also performed to find out whether the presence of 1/500 milk in the suspension protected the organisms and delayed the rate at which they were destroyed. In each experiment one suspension was made up with glass-distilled water

alone, the other with glass-distilled water containing a 1/5000 concentration of full-cream dried milk, which was approximately equivalent to a 1/500 concentration of ordinary liquid milk. The findings are recorded in Table 9. There was some variation in the values

Table 9. *Comparison of velocity constants for* NaOH *between suspensions of* Bact. coli *in distilled water and in* 1/500 *milk*

Date of exp.	Concentration and temperature	Time in min.	Mean value of k: Distilled water	Mean value of k: 1/500 milk
29. iii. 39	0·1 % NaOH at 20° C.	0–6	0·445	0·410
30. iii. 39	,, ,,	0–8	0·266	0·357
31. iii. 39	,, ,,	0–8	0·349	0·333
Arithmetic mean	,, ,,		**0·353**	**0·367**
29. iii. 39	0·025 % NaOH at 40° C.	0–6	0·381	0·182
30. iii. 39	,, ,,	0–4	0·088	0·100
31. iii. 39	,, ,,	0–8	0·191	0·209
3. iv. 39	,, ,,	0–6	0·076	0·060
Arithmetic mean	,, ,,		**0·184**	**0·138**

of k in different experiments, but on the whole the averages were fairly similar. With 0·1 % NaOH at 20° C. the average reaction velocity was slightly higher in the milk suspension, while with 0·025 % NaOH at 40° C. it was rather higher in the distilled water suspension. It may be concluded that the presence of milk in a *final* concentration of 1/1000 has little effect on the germicidal power of caustic soda. Had one of the phenol group of disinfectants been used, which coagulate albumin, there is little doubt that the presence of the milk would have slowed the reaction considerably.

Concentration coefficient n

In Table 10 the mean values have been worked out for the concentration coefficient n.

Table 10. *Mean values of concentration coefficient n for destruction of* Bact. coli *by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean n
0·05 % NaOH at 20° C.	6	0·054	2·77
0·1 % ,, ,,	7	0·367	
0·05 % NaOH at 30° C.	5	0·116	2·60
0·1 % ,, ,,	3	0·701	
0·025 % NaOH at 40° C.	6	0·236	(0·72)
0·05 % ,, ,,	4	0·389	
Arithmetic mean			**2·69**

The values of k at 40° C. were very irregular, and it is probable that the figure of 0·236 obtained with 0·025 % NaOH was too high. Omitting therefore these results, and taking only the findings at 20 and 30° C., it is seen that the average value of n was about 2·7. This means in effect that doubling the concentration of NaOH increases the reaction velocity by $2^{2·7}$, or approximately 6·5 times. Halving it, on the other hand, decreases it by the same amount. The importance of using as high a concentration as possible of caustic soda when the time factor is concerned is thus clearly demonstrated.

Temperature coefficient θ

In Table 11 the mean values of the temperature coefficient $\theta^{10° C.}$ are recorded. There was some variation under different sets of conditions. The value for the comparison between 30 and 40° C. is probably too high. The reason for this is discussed on p. 444. The average

value may be taken as about 2. In practice this means that raising the temperature 10° C. doubles the rate of destruction. Though this effect is not as great as that of doubling the concentration of disinfectant, it is nevertheless sufficient to justify the use of as high a temperature as possible during the actual disinfectant stage of bottle washing.

Table 11. *Mean values of temperature coefficient* $\theta^{10°\,C.}$ *for destruction of* Bact. coli *by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean θ^{10}
0·05% NaOH at 20° C.	6	0·054	2·15
„ „ 30° C.	5	0·116	
0·05% NaOH at 30° C.	5	0·116	(3·35)
„ „ 40° C.	4	0·389	
0·1% NaOH at 20° C.	7	0·367	1·91
„ „ 30° C.	3	0·701	
Arithmetic mean			**2·03**

2. Observations on spores of *B. subtilis*

Technique

A spore-bearing organism isolated from milk, which for the sake of convenience will be referred to as *B. subtilis* though its exact identity was never worked out, was selected because it was found possible to prepare from it a homogeneously distributed suspension of spores.

The growth from twelve 4-day agar slope cultures at 37° C. of this organism was washed off with glass-distilled water and treated for an hour in the mechanical shaker. The suspension was centrifuged at about 2500 r.p.m. for 7 min. to throw down aggregated particles. The supernatant fluid was withdrawn and heated to 60° C. for 90 min. A viable count was made by the roll-tube method, and the suspension was stored in an amber bottle in the ice-chest.

Counts were made every few days. The number of viable organisms fell fairly rapidly for the first few days, after which it remained almost constant, at about $10–15 \times 10^6$ per ml., during the next 2 months. The suspension was not used for disinfection experiments till it was over a fortnight old.

This suspension lasted for nearly the whole series of our observations. A fresh suspension, however, had to be made up for the last three experiments; it proved to be slightly stronger than the first.

The experiments with caustic soda were carried out in the same way as with *Bact. coli*. The suspension of spores was mixed with a 1/5000 dilution of full-cream dried milk in the proportion of 1/9, and this suspension was added to an equal quantity of the caustic soda solution under test. Counts were made by the roll-tube method as before. The average initial number of organisms exposed to the disinfectant was about 6×10^6 per ml., instead of about 50×10^6 per ml. with *Bact. coli*. This meant that observations could be made over a rather smaller range of the disinfection curve.

Results. Mean value of k, n, and θ

Reaction velocity k

The mean values of the reaction-velocity constants for different concentrations of caustic soda at different temperatures are recorded in Tables 12–17. It will be noted that some variation occurred in the values of k between different experiments carried out under

Table 12. *Mean values of reaction velocity constant k for* 5% NaOH *at* 30° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
28. ii. 39	0–60	5·8639	4·8814	0·016
3. iii. 39	0–60	5·7118	4·5353	0·020
7. iii. 39	0–60	5·7453	4·5575	0·020
10. iii. 39	0–60	5·7364	4·6101	0·019
Total	240	23·0574	18·5843	0·075
Arithmetic mean	60	5·7644	4·6461	**0·019**

Table 13. *Mean values of reaction velocity constant k for* 10% NaOH *at* 30° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
28. ii. 39	0–30	5·8639	4·0315	0·061
3. iii. 39	0–30	5·7118	3·9045	0·060
10. iii. 39	0–30	5·7364	3·7559	0·066
Total	90	17·3121	11·6919	0·187
Arithmetic mean	30	5·7707	3·8973	**0·062**

Table 14. *Mean values of reaction velocity constant k for* 2% NaOH *at* 50° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
24. ii. 39	0–30	5·6663	3·7745	0·063
28. ii. 39	0–30	5·8639	3·6258	0·075
3. iii. 39	0–30	5·7118	3·0253	0·090
7. iii. 39	0–20	5·7453	4·2644	0·074
Total	110	22·9873	14·6900	0·302
Arithmetic mean	27·5	5·7468	3·6725	**0·076**

Table 15. *Mean values of reaction velocity constant k for* 5% NaOH *at* 50° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
28. ii. 39	0–5	5·8639	4·3617	0·300
3. iii. 39	0–6	5·7118	3·2480	0·411
10. iii. 39	0–4	5·7364	3·8639	0·468
Total	15	17·3121	11·4736	1·179
Arithmetic mean	5	5·7707	3·8245	**0·393**

Table 16. *Mean values of reaction velocity constant k for* 1% NaOH *at* 70° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean k
21. iii. 39	0–4	5·8349	4·6484	0·297
22. iii. 39	0–6	5·6322	2·9494	0·447
23. iii. 39	0–4	5·7973	4·4440	0·338
24. iii. 39	0–5	5·6385	3·6902	0·390
27. iii. 39	0–4	5·7284	4·4713	0·314
28. iii. 39	0–4	5·7578	5·1055	0·163
30. iii. 39	0–4	6·1461*	5·5267	0·155
31. iii. 39	0–4	6·1075*	5·2863	0·205
Total	35	46·6427	36·1218	2·309
Arithmetic mean	4·4	5·8303	4·5152	**0·289**

* New spore suspension used.

Table 17. *Mean values of reaction velocity constant k for* 2% NaOH *at* 70° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean k
27. iii. 39	0–1	5·7284	4·8488	0·880
28. iii. 39	0–1	5·7578	5·2467	0·511
30. iii. 39	0–1	6·1461*	5·6271	0·519
31. iii. 39	0–2	6·1075*	4·6258	0·741
3. iv. 39	0–2	6·3474*	5·3118	0·518
Total	7	30·0872	25·6602	3·169
Arithmetic mean	1·4	6·0174	5·1320	**0·634**

* New spore suspension used.

designedly the same conditions. As with *Bact. coli*, this variation tended to be greater at the higher temperatures—40° C. with *Bact. coli* and 70° C. with *B. subtilis* spores. It is probable that at these temperatures the level was being approached at which the heat by itself was lethal, and that the rate of disinfection was therefore determined by the coagulative action of the heat as well as by the germicidal power of the disinfectant. At such a critical level, minute changes in temperature may well have brought about a result incommensurate with that caused by similar changes at lower levels, and so led to greater variation in the value of k than was expected.

This explanation is to some extent borne out by a study of Tables 11 (p. 442) and 21 (p. 447). It will be noticed that the value of $\theta^{10° C.}$ is considerably higher when the comparison is made between 30 and 40° C. with *Bact. coli* and between 50 and 70° C. with *B. subtilis* spores than at lower temperatures. Presumably some additional factor came into operation at the higher temperature to augment the germicidal action of the caustic soda.

The rates of disinfection under different conditions are represented graphically in Fig. 2. Comparison with Fig. 1 (p. 439) shows how much stronger concentrations of caustic soda were required at any given temperature to destroy *B. subtilis* spores than *Bact. coli*.

Table 18 confirms the findings with *Bact. coli*, recorded in Table 7, namely, that destruction of bacteria proceeded less rapidly at first than later.

With both organisms a definite lag was observable in the early stages of disinfection, after which the rate of destruction increased to an appreciable, and often considerable extent. Whether it would have fallen off again in the later stages it is impossible to say,

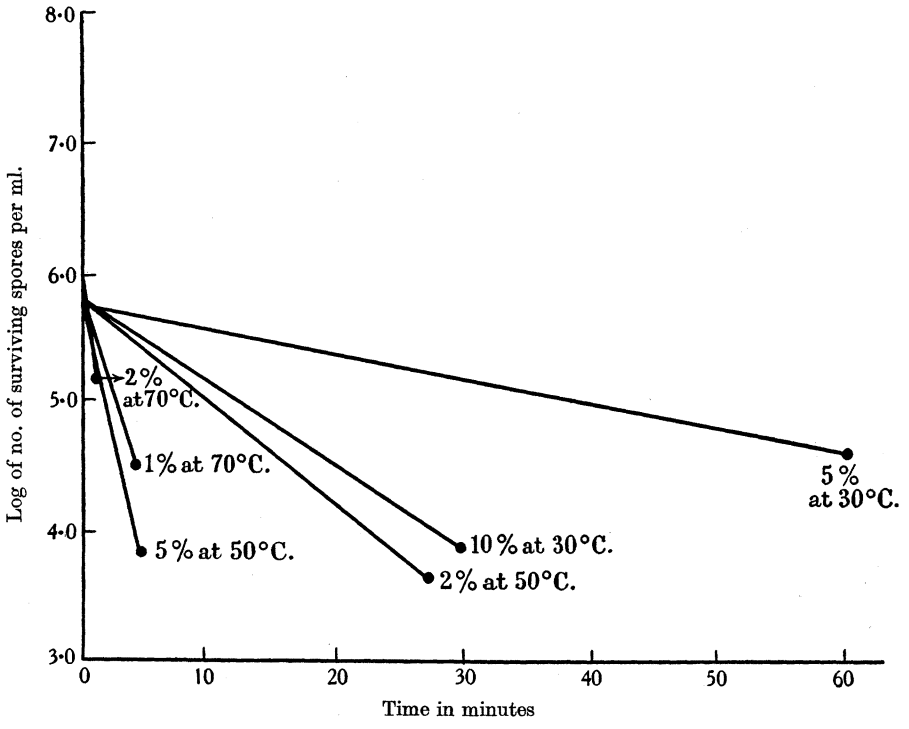

Fig. 2. Rate of death of spores of *B. subtilis* when submitted to different concentrations of sodium hydroxide at different temperatures.

Table 18. *Mean values of reaction velocity constants at different stages of the disinfection curve.* NaOH *with* B. subtilis *spores*

Concentration, temperature and no. of experiments (in brackets)	Above: Interval in min. Below: Mean *k*	
5% NaOH at 30° C. (4)	0–30 0·016	30–60 0·021
10% NaOH at 30° C. (3)	0–15 0·048	15–30 0·074
2% NaOH at 50° C. (3)	0–15 0·050	15–30 0·102
5% NaOH at 50° C. (2)	0–2 or 3 0·337	2 or 3–4 or 6 0·542
1% NaOH at 70° C. (6)	0–2 0·242	2–4 0·249
2% NaOH at 70° C. (2)	0–1 0·601	1–2 0·658

since technical difficulties prevented observations being carried out close to the sterility point.

A few experiments were carried out to see if the 1/500 concentration of milk in the bacterial suspension lowered the rate of disinfection by protecting the organisms against the caustic soda (Table 19).

Table 19. *Comparison of velocity constants for* NaOH *between suspensions of* B. subtilis *spores in distilled water and in* 1/500 *milk*

Date of exp.	Concentration and temperature	Time in min.	Mean value of k: Distilled water	Mean value of k: 1/500 milk
24. iii. 39	1% NaOH at 70° C.	0–5	0·218	0·390
31. iii. 39	,, ,,	0–4	0·097	0·206
Arithmetic mean	,, ,,		**0·158**	**0·298**
3. iv. 39	2% NaOH at 70° C.	0–2	**0·556**	**0·518**

With 2% NaOH at 70° C. the reaction velocity was rather greater in the distilled water than in the diluted milk suspension, but with 1% NaOH the reverse was observed. It is unfortunate that these comparisons were made at the high temperature of 70° C., since, as has already been pointed out, results tended to be more variable at 70° C. than at lower levels. If the results are taken in conjunction, however, with those obtained with *Bact. coli* (Table 9), it may be concluded that the presence of milk in a *final* concentration of 1/1000 does not have any appreciable protective action against caustic soda.

Concentration coefficient n

In Table 20 the mean values of the concentration coefficient n are recorded. The average value tends to be rather lower with *B. subtilis* spores than with *Bact. coli* (see

Table 20. *Mean values of concentration coefficient n for destruction of* B. subtilis *spores by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean n
5% NaOH at 30° C.	4	0·019	1·71
10% ,, ,,	3	0·062	
2% NaOH at 50° C.	4	0·076	1·79
5% ,, ,,	3	0·393	
1% NaOH at 70° C.	8	0·289	(1·13)
2% ,, ,,	5	0·634	
Arithmetic mean			**1·75**

Table 10). It will be noticed, however, that in each instance the values at the highest temperatures observed—70° C. with *B. subtilis* spores and 40° C. with *Bact. coli*—were less than at the lower temperatures. This suggests that at the higher temperatures some factor other than the mere difference in concentration was playing a part, and adds further support to the opinion already expressed that the heat by itself was beginning to exert a coagulative effect on the protein of the bacteria. If the figures at the higher temperatures are neglected, then the mean value of n for *B. subtilis* spores works out at 1·75. This means, in practice, that doubling the concentration of caustic soda increases the reaction velocity by $2^{1·75}$, or 3·4 times.

The mean value of n for *Bact. coli* and *B. subtilis* spores is 2·2. Doubling the concentration of caustic soda would therefore increase the reaction velocity by $2^{2·2}$, or 4·6 times. In other words, if with a given concentration and temperature of caustic soda sterility was reached in 9 min., then doubling the concentration would reduce the time to 2 min., while halving the concentration would increase it to about 40 min.

Temperature coefficient θ

Table 21 sets out the values of the temperature coefficient $\theta^{10° C.}$. For the reason already suggested, the value for $\theta^{10° C.}$ calculated from a comparison of the reaction velocities at 50 and 70° C. was probably too high. Insufficient figures are available to estimate an

Table 21. *Mean values of temperature coefficient* $\theta^{10° C.}$ *for destruction of* B. subtilis *spores by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean θ
5% NaOH at 30° C.	4	0·019	1·44
„ „ 50° C.	3	0·394	
2% NaOH at 50° C.	4	0·076	2·89
„ „ 70° C.	5	0·634	
Arithmetic mean			**2·17**

average value at lower temperatures, but it is probable that the real value was somewhere about 1·5. This is rather lower than that of 2·03 calculated for *Bact. coli* (see p. 442). It will be observed, in fact, that the effect both of increasing the concentration of disinfectant and of raising the temperature was less with *B. subtilis* spores than with *Bact. coli*. The explanation of this is not clear.

Comparison of k for Bact. coli *and spores of* B. subtilis

In Table 22 comparative values of the reaction velocity k for *Bact. coli* and for *B. subtilis* spores are recorded. It will be seen that at 30° C. the reaction velocity with 10% caustic soda acting on *B. subtilis* spores was only about one-half that of the 0·05% caustic

Table 22. *Summary of mean values of reaction velocity constants for destruction of* Bact. coli *and of* B. subtilis *spores by* NaOH

Bact. coli		*B. subtilis* spores	
Concentration of NaOH and temperature	Mean k	Concentration of NaOH and temperature	Mean k
0·05% at 20° C.	0·054	5% at 30° C.	0·019
0·05% at 30° C.	0·116	10% at 30° C.	0·062
0·025% at 40° C.	0·236	2% at 50° C.	0·076
0·1% at 20° C.	0·367	1% at 70° C.	0·289
0·05% at 40° C.	0·389	5% at 50° C.	0·393
0·1% at 30° C.	0·701	2% at 70° C.	0·634

soda solution acting on *Bact. coli*. Taking n for *Bact. coli* at 2·7 it follows that at the same concentration and temperature the cells of *Bact. coli* are destroyed over three million times as fast as the spores of *B. subtilis*.

DISCUSSION

The results we have obtained may be compared with those of recent workers. Levine, Buchanan & Lease (1926–7) and Myers (1929) studied the rate of disinfection of spores by different concentrations of caustic soda at different temperatures. Some of their figures may be compared with ours, though many are unsuitable for this purpose. Levine obtained a value of k for 2% NaOH at 50° C. of 0·069 as against our value of 0·076. Myers's value for 2% NaOH at 60° C. was 0·225. Taking $\theta^{10° C.}$ as 1·5, this gives a value for k at 50° C. of 0·15, which is considerably higher than that recorded by Levine or ourselves. Possibly a difference in the resistance of the spores may have been responsible for this

discrepancy. For n Myers obtained a value of 1·67 as against our 1·75. Our results therefore are in fairly good agreement with those of the American workers.

It is not our purpose here to discuss the physical factors underlying disinfection, but our results do have some bearing on the standardization of disinfectants. A review of the literature (see Topley & Wilson, 1936) suggests that when a homogeneous suspension of spores of approximately the same age is submitted to a germicidal agency the rate of destruction appears to be similar to that in a monomolecular reaction. When vegetative organisms of varying age are used, departures from this law are frequent, probably because the more recently generated organisms are less resistant than the older organisms.

In our experiments, both with vegetative bacteria and with spores, there was an unmistakable tendency for the velocity of the reaction to be lower in the earlier than in the later stages of disinfection. Observations were not made at sufficiently frequent intervals to decide whether there was merely an initial lag phase followed by a phase of destruction at a uniform rate, or whether the velocity of the reaction increased progressively.

Accurate estimations of the reaction velocity in the later stages of disinfection with a powerful germicidal agent are not easy to make. It might be thought that, provided the germicide in the mixture was adequately and rapidly neutralized before plating, regular results should be obtained, but in our experience this proved untrue. Not only were the counts far lower than was expected, but they were very erratic. If they had been accepted as correct the value of k in the later stages of disinfection would have increased progressively though irregularly. This may possibly be the explanation why both Levine *et al.* (1926–7) and Myers (1929) obtained a progressive increase in their reaction-velocity constants throughout the period of disinfection. Comparison of their values of k at the beginning and at the end of the disinfection period shows that the increase was far greater than in our own experiments, in which observations were limited to the early and middle phases of disinfection.

The variation in the value of k in a given experiment was in the experience of Levine and his co-workers so great that they came to regard the measurement of the reaction-velocity constant as unsuitable for determining the effects of concentration and temperature on the germicidal efficiency of caustic soda. They recommended instead that the comparison should be made on the basis of the time necessary to destroy 99·9% of the bacteria in the mixture. This conclusion is of obvious importance in the standardization of disinfectants. Though superficially attractive, it is open, in our opinion, to severe criticism. It implies that the time taken to kill 99·9% of the bacteria can be measured accurately. This we regard as extremely doubtful, for the reason advanced above. If a comparison is desired, we believe that the value of k taken in the middle stage of disinfection affords the most suitable measure. So long as the initial number of organisms is the same in each comparative experiment, the value of k during the middle stage of disinfection, or from the beginning of disinfection to about the end of the middle stage, constitutes an expression of the rate of disinfection that can be measured with reasonable accuracy. A comparison of the value of k for one disinfectant over a range of destruction from 10^7 to 10^3 organisms per ml. with another over a range from 10^7 to 10^6 organisms per ml. is not likely to reveal the true germicidal potency of the two reagents. Both should be compared over approximately the same range of destruction.

There is no need to enter more fully into this subject, since Withell (1942), in a paper written two to three years after our report was completed, has come to essentially the

same conclusions. His recommendation for comparing disinfectants is to choose the time necessary to destroy 50% of the organisms in the suspension. Such an end-point can be measured with considerable accuracy, and, though similar in principle, it has obvious practical advantages over the measurement of k in the middle stage of the reaction.

Finally, it is of interest to compare the germicidal activity of caustic soda with that of phenol. A comparison of our figures for caustic soda with those of Chick (1908) for phenol shows that a 0·05% solution of caustic soda at 20° C. destroyed *Bact. coli* about five times as fast as a 0·5% solution of phenol at 20° C. destroyed *Bact. paratyphosum* B, and that a 5% solution of caustic soda at 30° C. destroyed spores of *B. subtilis* nearly three times as fast as a 5% solution of phenol at 33·3° C. destroyed anthrax spores. Even allowing for the higher value of n for phenol—about 5 as opposed to 2·2 for caustic soda—the superiority of caustic soda at concentrations likely to be used in practice is very considerable.

SUMMARY

Quantitative observations were made on the rate of disinfection of *Bacterium coli* and of the spores of *Bacillus subtilis* by caustic soda at different temperatures and different concentrations. The tests were carried out in the presence of 1/1000 milk. It was found that:

(1) With *Bact. coli* the concentration coefficient n was about 2·7, and the temperature coefficient $\theta^{10° C.}$ about 2.

(2) With *B. subtilis* spores the concentration coefficient n was about 1·75 and the temperature coefficient $\theta^{10° C.}$ about 1·5.

(3) With *Bact. coli* the values for the reaction velocity constant k tended to be irregular at 40° C. and with *B. subtilis* spores at 70° C., suggesting that at these temperatures some additional factor, presumably heat coagulation of the protein, was beginning to affect the results.

(4) With both organisms the value of the reaction velocity constant k was relatively slow at the start and tended to increase progressively during the course of disinfection. Whether it diminished again as sterility was approached could not be ascertained for technical reasons.

(5) With both organisms the presence of 1/1000 milk did not seem to affect the rate of disinfection as compared with distilled water.

(6) One experiment with *Bact. coli* suggested that the rate of disinfection was affected appreciably by the number of organisms present. Increasing the number of organisms in the suspension 100 times diminished the value of k by about one-third.

(7) The value of k was about 3,000,000 times greater with *Bact. coli* than with *B. subtilis* spores.

(8) If the mean value of n is taken as 2·2, it follows that doubling the concentration of caustic soda increases the reaction velocity by 4·6 times. If, for example, with a given concentration of caustic soda sterility was reached in 9 min., then doubling the concentration would reduce this time to 2 min., while halving the concentration would increase it to about 40 min.

(9) If the mean value of $\theta^{10° C.}$ is taken as 1·75, it follows that a rise of 10° C. in the temperature increases the reaction velocity 1·75 times. If, for example, with a given temperature sterility was reached in 9 min., then raising the temperature 10° C. (18° F.)

would reduce this time to about 5 min., while lowering the temperature 10° C. would increase it to about 15 min. With temperatures, however, of over 40° C. (104° F.) the rate of destruction of vegetative organisms, and with temperatures of over 70° C. (158° F.) the rate of destruction of sporing organisms in the presence of caustic soda is probably increased by the effect of the heat itself.

(10) A comparison of our figures for caustic soda with those of Chick for phenol shows that a 0·05% solution of caustic soda at 20° C. destroyed *Bact. coli* about five times as fast as a 0·5% solution of phenol at 20° C. destroyed *Bact. paratyphosum* B, and that a 5% solution of caustic soda at 30° C. destroyed *B. subtilis* spores nearly three times as fast as a 5% solution of phenol at 33·3° C. destroyed anthrax spores. The superiority of caustic soda over phenol, particularly at concentrations likely to be used in practice, is manifest.

(11) Discussing the standardization of disinfectants, we conclude that the value of k taken in the middle stage of the reaction, or from the beginning of disinfection to about the end of the middle stage, affords the most suitable measure of comparison. This is essentially the same conclusion as that reached by Withell (1942), who uses as his index the time necessary to destroy 50% of the organisms.

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